Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo-Report of the investigation of **Syloid** in the developing chicken embryo
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February 28, 1974

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SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo

Attached is the report of the investigation of SYLOID in the developing chicken embryo.

Investigations of the Toxic and Teratogenic Effects of
GRAS Substances to the Developing Chicken Embryo:

SYLOID

PROTOCOL:

Syloid (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered, with water as the solvent by two routes and at two stages of embryonic development; via the albumen at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2, 3, 4).

The route of albumen, instead of the usual air cell, was chosen because of the fact that the administered syloid solution formed globular coagulates as soon as it was injected into the air cell, and absorption through the embryonic membrane was not conceivable.

Groups of ten or more eggs were treated under these four conditions at several dose levels until a suitable total number of eggs per level was reached for all levels allowing some to hatch. Groups of adequate size were treated solely with the solvent at corresponding volumes. Untreated controls were also included in each experiment.

After treatment, all the eggs were candled daily and the non-viable embryos were removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. Along with these, histological examinations of major organs (liver, heart, kidney, lung, brain, intestine, gonad, and some endocrine organs) were carried out by taking samples from a representative number of animals from each experimental group.

RESULTS:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of the test.

Columns 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram egg weight, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i. e., the total number of non-viable eggs divided by the total number of treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total number of eggs treated. This includes all the abnormalities observed and also the toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia, and neural disorders including ataxia.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as a percentage of the total number of eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Columns 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (5). The results obtained are indicated at the bottom of each table.

The data in columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

DISCUSSION:

Syloid was found to be embryotoxic when administered to the embryos under all conditions of the test. The toxicity was significantly (P=0.05) greater than in solvent-treated eggs at all dose levels tested except at 10 mg/egg via the albumen at both 0 hours and 96 hours. Probit analysis resulted in an LC_{50} of 7.412 mg/egg (albumen at 0 hours, Table 1) and an LC_{50} of 4.323 mg/egg (albumen at 96 hours, Table 2). Each yolk treatment at 0 and 96 hours resulted in a line whose slope was not significantly different from zero (Tables 3 and 4, respectively).

Abnormal birds were seen under all conditions of the test. In no case was the frequency of abnormalities significantly different from that of the solvent background (P=0.05). Six abnormal birds, all with curled toes, were produced by the 101 control eggs.

ALBUMEN AT 0 HOURS: At 10.0 mg/egg, six abnormal birds were found; three had hip contracture, two had curled toes and one had celosomia. At 5.0 mg/egg, eight birds with abnormalities were seen; two had hip contracture, five had curled toes and one had celosomia. At 1.0 mg/egg, six abnormal birds were seen; three had curled toes and the remaining three

had one of the following: hypopigmentation of the down, celosomia, shoulder contracture. At 0.5 mg/egg, three birds with curled toes and one bird with hip contracture were found. The solvent-treated controls produced five abnormal birds; two had hip contracture and three had curled toes.

ALBUMEN AT 96 HOURS: At 10.0 mg/egg, twelve abnormal birds were found; three had hip contracture, six had curled toes, two had celosomia and one had both a cardiomegaly and a small liver. At 5.0 mg/egg, one bird had hypopigmentation of the down, two had curled toes, one had hip contracture and one had celosomia. At 1.0 mg/egg, seven abnormal birds were found; four had curled toes and three had hip contracture. At 0.5 mg/egg, all four birds that were found to be abnormal had curled toes. The solvent-treated group had six abnormal birds; five had curled toes and one had shoulder contracture.

YOLK AT 0 HOURS: At 10.0 mg/egg, seven abnormal birds were found, three had celosomia, one had an abnormal curvature of the maxilla, one had hip contracture and two had curled toes. At 5.0 mg/egg, two had hip contracture and two others had either curled toes or celosomia. At 1.0 mg/egg all three of the abnormal birds had celosomia. At 0.5 mg/egg, one had celosomia and the other had curled toes. The solvent-treated controls had four abnormal birds, all with curled toes.

YOLK AT 96 HOURS: At 10.0 mg/egg, two birds had hip contracture and the other three had one of the following: abnormal curvature of the maxilla, curled toes, or celosomia. At 5.0 mg/egg, five birds, one with celosomia, two with hip contracture, one with an enlarged liver, and one with an abnormal curvature of the maxilla, were found. At 1.0 mg/egg, one bird with celosomia, one with curled toes, and one with hip contracture were found. At 0.5 mg/egg, two birds, one with curled toes and the other with hypopigmentation of the down, were seen. The solvent-treated controls had three abnormal birds, all with curled toes.

Histological examinations of the major organs revealed no evidence of consistent change due to either the dose level of the substance administered or the mode of treatment.

From these results it cannot be concluded that syloid is teratogenic. However, both albumen and yolk treatment at 96 hours showed an abnormality of the viscera (change in liver size) or hypopigmentation of the down which were not observed in the solvent-treated embryos.

1. Syloid, 244, (micro-sized synthetic silica), silica aerogel sample #10, Division code, SMR 3-890, GRACE, Division Chemical, Baltimore, Maryland 21226

2. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M.J., Mutchler, M.K. and Fitzhugh, O.G. Toxicol. Appl. Pharmacol. 5:760-770, 1963

3. Verrett, M.J., Marliac, J.-P. and McLaughlin, J., Jr. JAOAC 47: 1002-1006, 1964

4. Hanan, E.B., Am. J. Anat. 38: 423-450, 1927

5. Finney, D.J. Probit Analysis, 2nd ed., Cambridge Press, Cambridge, Appendix I, 1964

Table 1

Syloid

Albumen at 0 Hours

Dose		Number	Percent	Percent Abnormal	
mg/egg	mg/kg	of eggs	Mortality	Total	Structural
10.0	200	91	73.62*	8.79	6.59
5.0	100	89	61.79*	10.11	8.98
1.0	20	88	55.68*	9.09	5.68
0.5	10	66	34.84	6.06	6.06
Water		85	38.82	7.05	5.88
Control		101	14.85	5.94	5.94

LC_{30} 2.340 mg/egg (46.813 mg/kg)

LC_{50} 7.412 mg/egg (148.245 mg/kg)

LC_{90} 123.996 mg/egg (2479.932 mg/kg)

*Significantly different from solvent $P \leq 0.05$

Table 2

Syloid

Albumen at 96 Hours

Dose		Number	Percent	Percent Abnormal	
mg/egg	mg/kg	of eggs	Mortality	Total	Structural
10.0	200	90	76.66*	14.44	13.33
5.0	100	99	59.59*	9.09	4.04
1.0	20	97	57.73*	7.21	7.21
0.5	10	66	31.81	7.57	6.06
Water		87	28.73	6.89	6.89
Control		101	14.85	5.94	5.94

LC_{30} 1.304 mg/egg (26.083 mg/kg)

LC_{50} 4.323 mg/egg (86.477 mg/kg)

LC_{90} 80.914 mg/egg (1618.289 mg/kg)

*Significantly different from solvent $P \leq 0.05$

Table 3

Syloid

Yolk at 0 Hours

Dose		Number	Percent	Percent Abnormal	
mg/egg	mg/kg	of eggs	Mortality	Total	Structural
10.0	200	129	67.44*	6.97	5.42
5.0	100	129	68.99*	4.65	3.10
1.0	20	129	64.34*	3.87	2.32
0.5	10	129	63.56*	1.55	1.55
Water		129	10.07	3.10	3.10
Control		101	14.85	5.94	5.94

F (calculated) $<$ F (0.05)

*Significantly different from solvent $P \leq 0.05$

Table 4

Syloid

Yolk at 96 Hours

Dose		Number	Percent	Percent Abnormal	
mg/egg	mg/kg	of eggs	Mortality	Total	Structural
10.0	200	135	72.59*	5.18	3.70
5.0	100	124	62.90*	6.45	4.03
1.0	20	124	52.41*	3.22	2.41
0.5	10	125	58.40*	1.60	0.80
Water		125	8.00	2.40	2.40
Control		101	14.85	5.94	5.94

F (calculated) $<$ F (0.05)

*Significantly different from solvent $P \leq 0.05$